Simple determination of East Asian Y chromosomal haplogroups using multiplex allele-specific PCR assay

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Introduction

Y-chromosomal haplogroup which is defined by the combination of allelic states at hierarchically arranged Y-SNPs and small indels has been studied to infer the origins, evolution, and histories of migrations of modern human populations. Recently, newly revised Y-haplogroup tree containing 311 distinct haplogroups was published (Genome Res 18:830-8) and its resolution was increased. Especially haplogroup O, a major haplogroup in East Asians was considerably rearranged. Therefore, more efficient Y-SNP genotyping methods for convenient determination of East Asian Y-haplogroups should be developed according to the revised Y-haplogroup tree. Several techniques have been proposed to assess the genotypes of Y-SNP markers and single base extension (SBE) method is mainly used in forensic genetic studies. However, the need for rapid, simple and reliable methods for scoring large numbers of samples is increasingly growing. Here we described the development of multiplex allele-specific PCR (AS-PCR) system for simultaneous detection of Y-SNPs followed by fragment analysis on an automatic DNA sequencer like general forensic STR typing method.

Materials and Methods

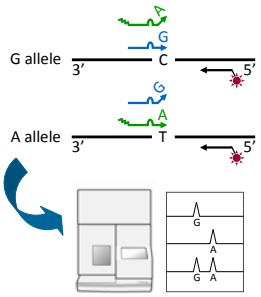
Samples

DNA samples from 300 unrelated Korean males were obtained from the National Biobank of Korea. The DNA samples were already typed for Y-SNP markers by SBE reactions. Serially diluted DNA samples (1 ng, 500 pg, 250 pg, 125 pg, 62 pg and 31 pg) of Quantifiler Human DNA standard (Applied Biosystems, Foster City, CA, USA) were used to detect sensitivity of a multiplex allele-specific PCR.

Y chromosomal SNP selection

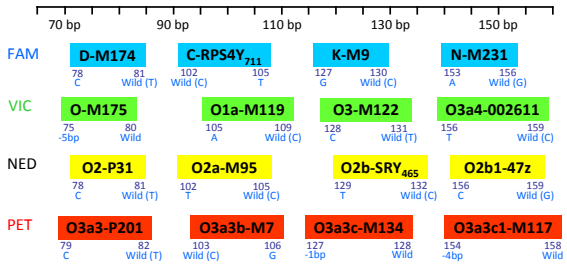
A set of 16 Y-chromosomal SNPs (M174, RPS4Y$_{711}$ (M130), M9, M231, M175, M119, P31, M95, SRY$_{465}$ (M176), 47z, M122, P201, M7, M134, M117 and JST002611) were selected to hierarchically designate haplogroups D, C, K, N, O, O1a, O2, O2a, O2b, O2b1, O3, O3a3, O3a3b, O3a3c, O3a3c1, and O3a4 for the identification of Y-haplogroups frequent in East Asians

Strategy for allele-specific PCR



Two allele-specific primers and a shared fluorescence-labeled primer were designed for each Y-SNP using the BatchPrimer3 program (http://wheat.pw.usda.gov/demos/BatchPrimer3/). The 3' end of an allele-specific primer was specific to one of two alleles of a SNP, and an additional mismatch was included at the third or other position from the 3' end in order to enhance the specificity in the allele-specific PCR reaction. One of the two allele-specific primers was modified to have a tail at its 5' end, thereby allowing different alleles to produce amplicons of different sizes.

Schematic of multiplex allele-specific PCR



Designed to produce small amplicons less than 160 bp

Multiplex allele-specific PCR amplification

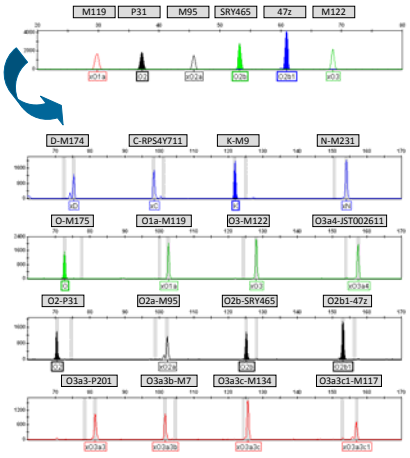
PCR reaction: Total 10 µl reaction contained 1 ng of template DNA, 1.0 µl of Gold ST*R 10X buffer (Promega, Madison, WI, USA), 2.5 U of AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA, USA) and each appropriate concentration of primers except for 47z marker. For 47z marker, monoplex PCR was performed under the same condition as above except the use of 0.5 U of AmpliTaq Gold® DNA polymerase (Applied Biosystems).

Cycling condition: 95˚C for 11 min; 30 cycles of 94˚C for 20 sec, 59˚C for 1 min, and 72˚C for 30 sec; and a final extension of 60˚C for 45 min using a Veriti 96-well thermal cycler (Applied Biosystems)

Detection system: ABI PRISM 310 Genetic Analyzer, GeneScan software 3.7 and GeneMapper ID 3.2 software (Applied Biosystems)

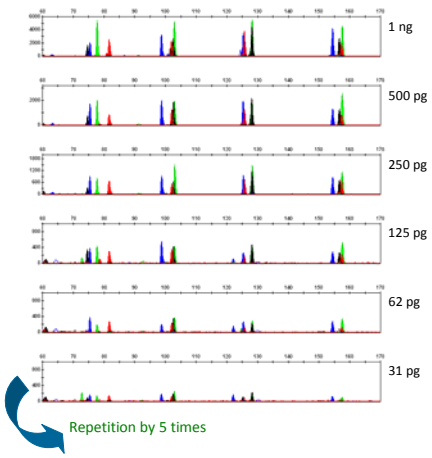
Results

Fig. 1. Concordance test between multiplex AS-PCR assay and multiplex SBE reaction



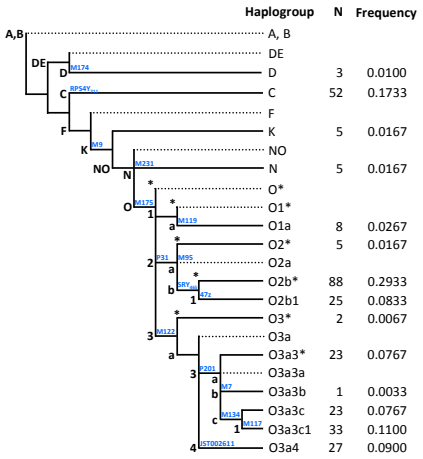
No difference was found between multiplex AS-PCR and multiplex SBE reaction of the Y-SNP genotyping results in 300 Korean males

Fig. 2. Sensitivity test with a serially diluted human DNA standard



Reliable results were obtained using as low as 250 pg of DNA with no allelic drop-in or drop-out. Pseudo-positive allele began to appear at 125 pg and allelic drop-out was shown below 62 pg.

Fig. 3. Phylogenetic tree of the 16 Y-chromosomal binary polymorphisms and with their corresponding haplogroup frequencies observed in 300 Korean males



14 Y-haplogroups were identified and haplogroup diversity was 0.8461.

Conclusion

- **A multiplex allele-specific PCR system was developed for simple, rapid and reliable scoring of Y-chromosomal SNPs to determine Y-haplogroups frequent in East Asians.**
- **The multiplex allele-specific PCR assay was optimized for simultaneous detection of 16 SNPs followed by fragment analysis on an automatic DNA sequencer like general forensic STR typing method.**
- **In sensitivity test, the multiplex allele-specific PCR system showed good performance with as low as 250 pg of DNA while showing no pseudo-positive result or allelic drop-out.**
- **A total of 14 different Y-haplogroups were identified using the developed multiplex allele-specific PCR in 300 Koreans; haplogroup O2b* was most frequently observed (29.3%), followed by haplogroup C (17.3%) and O3a3c1 (11.0%). The haplogroup diversity was 0.8461.**

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